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The concentrations of several organochlorines (OCs) were measured in the milk of five healthy bottlenose dolphins, *Tursips truncatus*, of various ages and reproductive histories. These dolphins were trained to position at penside to allow for milk collection; samples were collected at various stages of lactation. The highest values in ppm wet weight (lipid weight in parentheses) were: 10.2 (32.4) p,p'-DDE, 0,586 (1.86) p,p'-DDD, 0.251 (0.797) p,p'-DDT and 4.47 (14.1) PCB (as Aroclor 1254), and were found in milk produced by the oldest dolphin (age 34) during what was probably its first, and orphan-induced, lactation. The lowest values recovered were: 0.330 (1.62) p,p'-DDE, 0.027 (0.132) p,p'-DDD, <0.025 p,p'-DDT and 0.0281 (1.38) PCB, found in milk produced by one of the youngest dolphins (age 16). These data suggest that OCs may be released at levels related to age, reproductive history and lipid content of milk. The collection technique used allows for a serial study without animal injury or mortality.

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# Residue Levels of Several Organochlorines in *Tursiops truncatus* Milk Collected at Varied Stages of Lactation

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**The concentrations of several organochlorines (OCs) were measured in the milk of five healthy bottlenose dolphins, *Tursiops truncatus*, of various ages and reproductive histories. These dolphins were trained to position at penside to allow for milk collection; samples were collected at various stages of lactation. The highest values in ppm wet weight (lipid weight in parentheses) were: 10.2 (32.4) *p,p'*-DDE, 0.586 (1.86) *p,p'*-DDD, 0.251 (0.797) *p,p'*-DDT and 4.45 (14.1) PCB (as Aroclor 1254), and were found in milk produced by the oldest dolphin (age 34) during what was probably its first, and orphan-induced, lactation. The lowest values recovered were: 0.330 (1.62) *p,p'*-DDE, 0.027 (0.132) *p,p'*-DDD, <0.025 *p,p'*-DDT and 0.0281 (1.38) PCB, found in milk produced by one of the youngest dolphins (age 16). These data suggest that OCs may be released at levels related to age, reproductive history and lipid content of milk. The collection technique used allows for a serial study without animal injury or mortality.**

Many organochlorines (OCs) are chemically and biologically stable and lipophilic—properties that result in their accumulation up the food chain. Dolphins feed high in the food chain, have relatively long life spans, and the blubber layer that is so important for buoyancy, nutritional storage and temperature regulation is an ideal repository for the accumulation of pollutants. Some of the highest concentrations found in any animal from the natural environment have been found in bottlenose dolphins (O'Shea *et al.*, 1980).

Detection of OCs in the tissues of small cetaceans was first reported in 1967 (Holden & Marsden, 1967). In 1978 Risebrough wrote 'Research undertaken to date indicates that no marine mammal anywhere in the world is presently without a body burden of a variety of synthetic organic compounds which did not exist in the environment prior to their creation by man'. Historically, the highest levels of OCs in marine mammals have been found in blubber, liver and milk (Addison & Brodie, 1977). In many of these species, OC levels increase in males with age yet decrease or plateau in reproductively active females (cf. Addison & Brodie, 1977, 1987; Gaskin *et al.*, 1983; Clausen & Andersen, 1988). Transplacental transfer to the foetus has been shown in the harbour seal, *Phoca vitulina* (Duinker & Hillebrand, 1979), and the fur seal, *Callorhinus ursinus* (Anas & Wilson Jr, 1970). However, Anas & Wilson Jr (1970) found that OC levels were much higher in the milk of the fur seal than in the foetus, thereby showing that lactation is the main route of maternal transfer of OCs to the offspring.

During lactation, OCs are transferred to the calf (Addison & Brodie, 1987), thus lowering maternal levels. It is only through the course of lactation that much of the body burden from females is reduced (Kurzel & Cetrulo, 1985). The transfer of OC residues to offspring explains why these levels do not continue to increase in the reproducing females of many marine mammal species, as they do in the males.

Reports of OC levels found in milk are rare, and usually come from nutritionally compromised and/or dead animals. These values often represent a single point in lactation. Cockcroft *et al.* (1989) proposed that lactating females impart the majority of their OC load during the first 7 weeks of lactation; however, without serial data representing samples from live animals confirmation of this theory is difficult.

The dolphins in our care have been trained for several husbandry behaviours that facilitate medical care. For example, lactating dolphins are trained for milk collection. This voluntary behaviour has enabled us to collect milk samples for OC analysis representative of different stages of lactation. For the first time, it has been possible to monitor the transfer of OCs over time via milk collection, and without the capture or death of the animal.

## Materials and Methods

*Dolphin subjects*

All dolphin subjects had been originally collected in the Gulf of Mexico or were born at our facility from parents collected there.

*TOD*: On 12 November 1992, a 7-month-old, male bottlenose dolphin calf was orphaned. He was given a companion, TOD, a 34-year-old female bottlenose dolphin. TOD has lived at our facility for 26 years. Progesterone testing during semi-annual physicals never indicated pregnancy. The orphan began to nurse from TOD; 5 days after the initial introduction we suspected that TOD was lactating and on day eight collected the first milk. This induced lactation continued for about 455 days.

*SLA*: On 19 October 1992, a 6-month-old, male bottlenose dolphin calf was orphaned. He was housed with SLA, a 32-year-old female bottlenose dolphin who had successfully produced and nursed a calf, SAY, who was weaned in 1981. Since that time, annual physicals including progesterone testing confirmed that she was not pregnant. The orphaned calf was seen attempting to nurse from SLA, and by the 7th day it was suspected that she was lactating. A milk sample was collected on the 10th day (Ridgway *et al.*, 1993); relactation continued for about 301 days.

*SAY*: SAY, offspring of SLA, was born at our facility in 1979. At the age of 12 years, SAY gave birth for the first time. All three generations still live together in the same enclosure. SAY was the first dolphin trained for milk collection and the animal from which milk has been collected over the longest period of time.

*BER*: In 1993, 32-year-old BER gave birth for the first time since she was collected from the Gulf of Mexico in 1972. Because of her attentiveness to the calf, the first milk sample from BER was not collected until 64 days after its birth.

*ELL*: In 1992, 16-year-old ELL gave birth for the first time since her arrival at our facility in 1984. Milk samples were collected on days 169 and 180 of lactation.

Milks from two other dolphins, COR (age 7) and PNN (age 13) were collected soon after parturition. The calf of COR died 5 days after birth and a milk sample was collected 3 days later. The calf of PNN was stillborn and a sample was collected 21 h later. These samples were analysed for lipid content only.

Dolphins were conditioned to position ventrally at penside for milk collection (Fig. 1(a)). The mammary slits were rinsed with distilled water and excess water was removed. Milk was collected (Fig. 1(b)) using a breast pump* modified to allow for the morphological differences of each animal (Fig. 1(c)). Milk samples were collected from SAY, ELL and BER when possible after the births of their calves and at different stages of lactation. Once lactation was suspected in SLA and TOD, milk samples were successively collected during the initial stages of lactation; collections were continued as possible throughout lactation. This was the first record of induced lactation in bottlenose dolphins (Ridgway *et al.*, 1993).

In 'normally' lactating bottlenose dolphins (those lactating after pregnancy), the likelihood of collecting a milk sample during the first few months after the birth of a calf is low because the mother is not likely to leave her calf to volunteer for sampling. This is because the calf initially remains close by the mother's side almost continuously. As it gets older, the calf becomes more independent. Only when the calf begins leaving the mother to swim on its own can milk collection begin. This was the case with BER, ELL and SAY, however, the orphaned calves with TOD and SLA were older and we were able to collect milk samples from the first couple of weeks of induced lactation. Collection throughout lactation allowed serial analysis of milk samples (Table 1). Because samples collected on some days were not large enough to perform all analyses, we sometimes pooled samples from up to 7 days. To compare the lipid composition of the milk from the early stages of a nomal and an induced lactation, lipid analysis was performed on a sample collected from COR on the 8th day of lactation after she lost her calf, and on a sample collected from PNN 21 h after she gave birth to a dead calf. Because of the small size of these samples, organochlorine analysis could not be performed.

*Diet*

Daily, each dolphin was fed a variety of fish that had been fresh frozen and thawed. Food for all dolphins was from the same lots and included herring (*Clupea pallasi* or *Clupea harengus*), Pacific mackerel (*Scomber japonicus*), capelin (*Mallotus villosus*), squid (*Loligo opalescens*), and Columbia River smelt (*Thaleichthys pacificus*). Random samples of each new lot of each species of fish were sent to a laboratory for proximate analysis for nutritional assessment, but not OC content. The dolphins were housed in netted ocean enclosures located in San Diego Bay, 32°42'N/117°14'W. Although the nets allow small fish to swim through, any live fish caught and eaten by the dolphins were an insignificant addition to their diets. We did not analyse food fish for OCs because of the large numbers of species, lots and samples required, and because the animals' OC burden would have been accumulated from food consumed over many years prior to this study. It would be difficult to evaluate the contribution of the current diet to the milk residues.

*Analysis methods*

Samples were decanted into 4 ml aliquots, put into vials and frozen at −70°C for storage until sent to the laboratory† for analysis. For a control, a sample of pasteurized whole bovine milk was poured into one of the collection vials; another was pumped through the breast pump into the collection vial. Neither of these samples showed detectable levels of any of the measured contaminants. The following sections outline methods of analysis developed by the laboratory.

*Fat* was hydrolyzed on a water bath using hydrochloric acid. The lipid was extracted from milk samples using ether and hexanes. The extract was then dried and

*LOPUCO Ltd, 1615 Old Annapolis Road, Woodbine, MD 21797, USA.

†Hazleton Wisconsin Laboratories, 3301 Kinsman Blvd, Madison, WI 53707, USA.

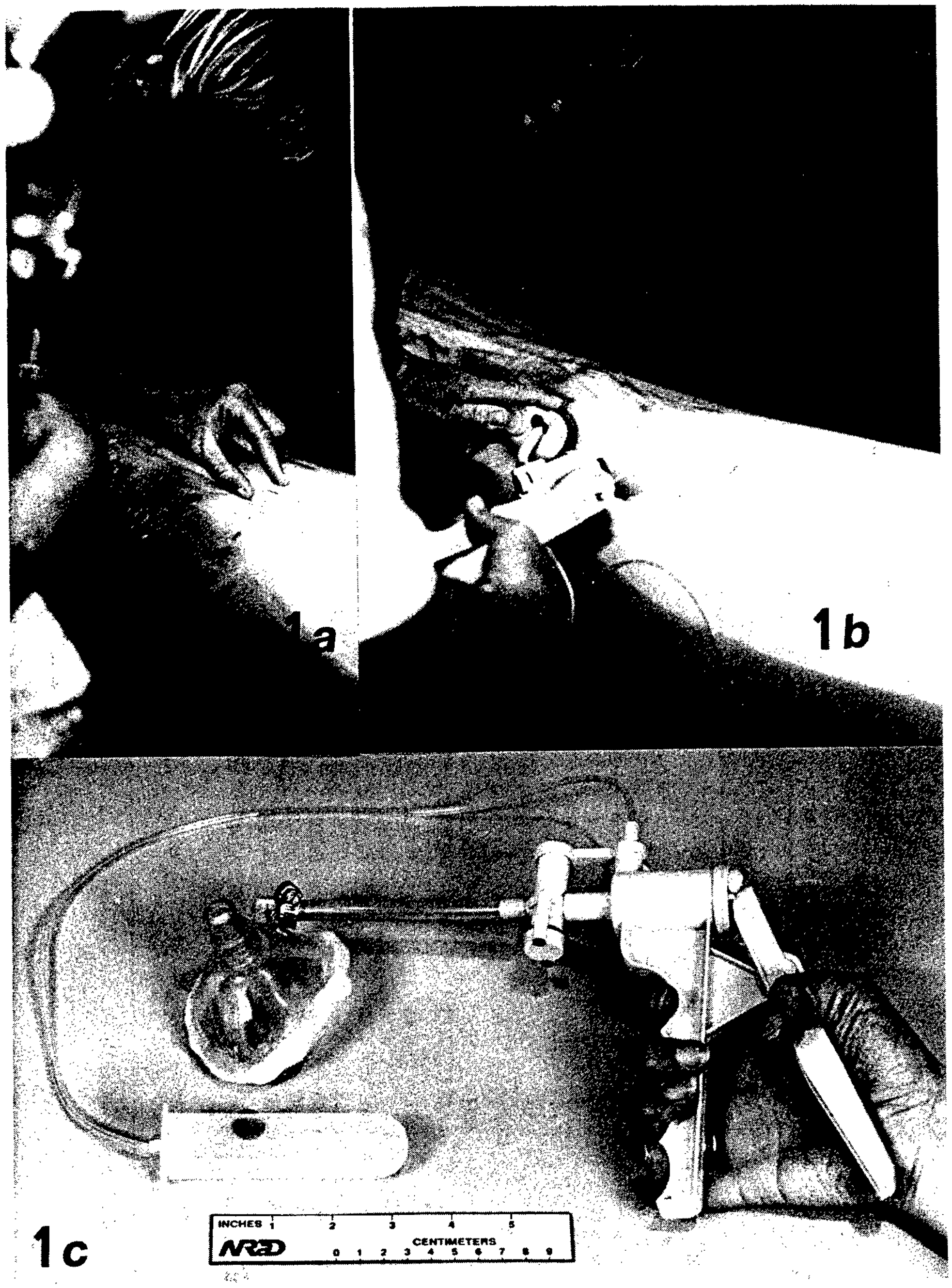


**Fig. 1** (a) Once the dolphin has positioned itself ventrally at penside, the mammary slits are rinsed with distilled water and dried. (b) A modified breast pump is placed over one of the mammary slits and a sample is collected. (c) Hand operated vacuum pump modified for use for collection of dolphin milk samples.

weighed. (Method 922.06, 954.02 (modified) of the AOAC (1990).)

For *organochlorines*, samples were dried with sodium sulphate, extracted with ethyl acetate and concentrated. Lipids were removed using gel permeation chromatography (GPC). Fractions containing chlorinated pesticides and PCBs were collected, and cleaned up for PCBs and other chlorinated compounds using Florisil column chromatography. Chlorinated compounds were identified by gas chromatography using electron capture detection (GC/ECD) and electrolytic conductive detection (GC/ELCD). PCB isomers were quantified as Aroclor 1254 (*Journal of the Association of Official Analytical Chemists* (JAOAC), 1965, 1976; *Pesticide Analytical Manual* (PAM), 1968).

*Blubber measurement*

To monitor body condition during lactation, we measured the blubber thickness using a portable ultrasound machine, SCANPROBE.* Measurements were taken from an area just anterior to the insertion of the dorsal fin. This site has been shown to adequately monitor changes of blubber thicknes for each individual animal (T. Williams, pers. comm.).

*SCANCO Incorporated, 2776 N. Triphammer Road, Ithaca, NY 14850, USA.

**TABLE 1**

Organochlorines in the milk of five bottlenose dolphins (ppm, $\mu g\ g^{-1}$).

| | | | | | PCB | | $p,p'$-DDE | | $p,p'$-DDD | | $p,p'$-DDT | | Dieldrin | | HCB | | Heptachlor epoxide | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Detection limit (wet wt) | | | | | 0.250 | | 0.0125 | | 0.0185 | | 0.0250 | | 0.0125 | | 0.00650 | | 0.0125 | |
| Name/ age | F/NF | Day | Blubber (mm) | Lipid (%) | Wet | Lipid | Wet | Lipid | Wet | Lipid | Wet | Lipid | Wet | Lipid | Wet | Lipid | Wet | Lipid |
| TOD 34 years | NF | 8 | NM | 6 | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM |
| | NF | 11–18 | NM | 8 | 1.00 | 12.9 | 2.00 | 26.0 | 0.103 | 1.34 | ND | ND | ND | ND | ND | ND | ND | ND |
| | NF | 68, 71 | 34 | 24 | 1.11 | 4.72 | 3.10 | 13.2 | 0.215 | 0.915 | ND | ND | 0.0138 | 0.0587 | ND | ND | 0.0190 | 0.08 |
| | NF | 127, 138 | 34 | 27 | 2.74 | 10.3 | 3.39 | 12.8 | 0.194 | 0.732 | 0.101 | 0.381 | 0.0360 | 0.136 | 0.0100 | 0.0377 | 0.0210 | 0.07 |
| | F & NF | 265–293 | 34 | 32 | 4.45 | 14.1 | 10.2 | 32.4 | 0.586 | 1.86 | 0.251 | 0.797 | 0.0768 | 0.244 | 0.0319 | 0.101 | 0.0397 | 0.12 |
| SLA 32 years | NF | 10 | NM | 10 | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM | NM |
| | NF | 79–100 | 35 | 20 | 0.634 | 3.17 | 0.427 | 2.14 | 0.056 | 0.280 | 0.0384 | 0.192 | ND | ND | ND | ND | ND | ND |
| SAY 12 years | NF | 94–145 | NM | 22 | 0.878 | 4.01 | 1.85 | 8.45 | 0.106 | 0.484 | 0.0667 | 0.305 | 0.0172 | 0.0785 | 0.0109 | 0.0498 | ND | ND |
| | NF | 375–383 | NM | 24 | 0.680 | 2.81 | 1.15 | 4.75 | 0.071 | 0.293 | 0.0512 | 0.212 | 0.0125 | 0.0517 | 0.00853 | 0.0352 | ND | ND |
| | F | 374–383 | NM | 23 | 0.595 | 2.56 | 1.09 | 4.70 | 0.070 | 0.300 | 0.0536 | 0.231 | 0.0132 | 0.0569 | 0.00987 | 0.0425 | ND | ND |
| | F | 431–437 | NM | 25 | 0.655 | 2.64 | 0.925 | 3.73 | 0.057 | 0.229 | 0.0419 | 0.169 | 0.0132 | 0.0532 | ND | ND | ND | ND |
| | NF | 502–509 | NM | 24 | 0.334 | 1.38 | 0.425 | 1.76 | 0.026 | 0.108 | ND | ND | ND | ND | ND | ND | ND | ND |
| | NF | 592 | 35 | 21 | 0.284 | 1.35 | 0.430 | 2.05 | 0.034 | 0.162 | 0.0281 | 0.134 | ND | ND | 0.0105 | 0.0500 | ND | ND |
| | F | 615 | 38 | 26 | 0.334 | 1.26 | 0.400 | 1.51 | 0.034 | 0.129 | 0.0289 | 0.109 | ND | ND | 0.0107 | 0.0404 | ND | ND |
| BER 32 years | NF | 112–114 | 32 | 14 | 1.10 | 7.75 | 2.13 | 15.0 | 0.233 | 1.64 | 0.0728 | 0.513 | 0.0266 | 0.187 | 0.0128 | 0.0901 | ND | ND |
| ELL 16 years | F & NF | 169–180 | NM | 20 | 0.281 | 1.38 | 0.330 | 1.62 | 0.027 | 0.132 | ND | ND | ND | ND | ND | ND | ND | ND |

Wet weight and lipid weight (F = fasting, NF = non-fasting, NM = not measured, ND = not detected).

## Results and Discussion

Results on a wet weight basis from the OC analyses are presented in Table 1. The highest values for DDT and PCB residues were found in milk produced by TOD, the oldest dolphin who has not calved in at least 26 years, if ever. Generally, as lipid levels increased from day 11 to day 293 (Table 1), so did levels of PCB and *p,p'*-DDE. Levels of *p,p'*-DDT and HCB were not detectable in the first two analyses, but were detectable in increasing levels in the samples collected from days 127 to 293. Overall, DDD increased from 0.103 ppm wet wt (1.34 ppm lipid weight), on days 11–18 to 0.586 ppm wet wt (1.86 ppm lipid weight) on days 265–293.

The lowest values were from milk produced by ELL, one of the youngest dolphins. Also showing lower levels were the samples collected from SLA. Compared to TOD, the OC values in SLA's milk were much lower, even though the lipid content of the milk and the animals' ages are comparable. A notable difference between these two dolphins is the fact that this was probably the first lactation for TOD, and the second for SLA. The lower levels of OCs in SLA's milk are probably reflective of lower levels in her body, since SLA had lactated 12 years earlier for a period of 2 years from 1979 to 1981 while nursing her calf SAY. During this first lactation, OCs presumably were transferred in the milk of SLA to SAY, thereby reducing body levels in SLA. The single analysis on SLA contained lower levels of all OCs than the first two samples from SAY, even though the milk lipid levels were similar. SAY's milk lipid only varied from 21 to 26% during more than 500 days of the lactation period represented by these data. Levels of PCB and *p,p'*-DDE declined markedly during this period (Fig. 2) as did *p,p'*-DDT, while HCB, heptachlor epoxide and dieldrin remained unchanged. The fact that this animal was born at our facility may be one possible explanation for the decrease in OCs in the milk while percent lipid remained consistent. Most of the food items fed to the animals in our facility are mainly pelagic and usually found offshore, away from the coastal origins of most OC contamination.

A single high dose of a toxin increases the likelihood of biological defects (Kurzel & Cetrulo, 1985). Of the milk analysed for OCs in our study, the lowest percent lipid (8%) was found in TOD's milk between day 11 and 18. This sample represented the earliest sample for which we were able to run OC analysis. This first sample also had the lowest levels of OCs found which continued to increase as lipid content increased in TOD's milk (Table 1). During this period of over 280 days the animal's body condition, as measured by blubber thickness, remained constant. However, the lipid content in the first milk sample from TOD (6%) was much lower than that found in milk for COR (19%) or PNN (21%), suggesting that milk from orphan-induced lactation does not initially have the same lipid content as milk from 'normal' lactation that follows pregnancy.

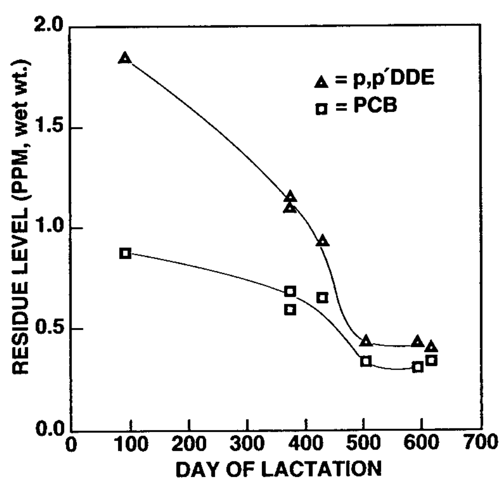


**Fig. 2** Residue levels of PCB and *p,p'*-DDE in SAY's milk collected from day 94 to day 615.

Our data from bottlenose dolphins is consistent with several past studies which suggest that the concentration of OC residues varies with physiological factors such as age and reproductive history (Addison & Brodie, 1977, 1987; Duinker & Hillebrand, 1979; Addison, 1989). We have provided more specific data and our study is the first to include serial data from the same animal.

We are now gaining a better understanding of the rate and route of maternal transfer of OCs to offspring. However, the effects of these persistent pollutants in marine mammals are still poorly understood. Observations on known individuals with documented health histories over long periods of time should increase our understanding of the effects of these pollutants.

For training the milking procedure and collecting the milk samples, we thank the trainers at the Naval Command Control and Ocean Surveillance Center, RDT&E Division, especially Patricia Kamolnick, Lauryn Crosthwaite and Janet Hendrickson. We thank Marine World Africa, USA, for COR's milk sample. We thank Rhonda Gulbranson and Fritz Keller at Hazleton for technical support, and Dr Raymond J. Tarpley from Texas A&M, and John Frazier and Scott Echols from Northwestern Aquatic Sciences, for reading the manuscript. This work was supported by the Strategic Environmental Research and Development Program (SERDP).

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